EXERCISE INTERVENTION TO IMPROVE GLUCOSE TOLERANCE

by

Kyle William Wehmanen

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

Department of Exercise and Sport Science

The University of Utah

December 2014

The University of Utah Graduate School

STATEMENT OF THESIS APPROVAL

The thesis of **Kyle William Wehmanen**

has been approved by the following supervisory committee members:

James C. Martin	, Chair	**10/27/14** Date Approved
Russell S. Richardson	, Member	**10/27/14** Date Approved
Timothy E. Graham	, Member	**10/27/14** Date Approved

and by **Janet M. Shaw** , Chair

the Department of **Exercise and Sport Science**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

In 2012 over 27 million Americans were suffering from Type II Diabetes (T2DM), which cost the U.S. $245 billion dollars in health care costs. Luckily, lifestyle changes and exercise can slow or prevent development of the disease. The purpose of our study was to determine the effect of high-intensity single-leg cycle training on insulin sensitivity and blood glucose levels in individuals with metabolic syndrome and insulin resistance, which put them at risk of developing T2DM. Seven individuals (age: 43 ± 14 yrs, height: 165.0 ± 11.6 cm, weight: 103.6 ± 17.7, BMI: 38.1 ± 5.6 kg/m^2) who displayed insulin resistance (CLIX-IR: 3.3 ± 1.2) and metabolic syndrome completed 12-14 sessions of high-intensity, interval based, single-leg training (HIIT) at a minimal intensity of 80% of VO_{2PEAK} power. Pre- and postoral glucose tolerance, blood lipid, and VO_{2PEAK} tests were performed to determine the effect of training on insulin resistance, blood glucose levels, and additional health parameters. One-way repeated measure ANOVAs showed that single-leg training significantly improved HbA1c levels (5.8 ± 0.5% pretraining vs. 5.6 ± 0.4% posttraining; $p \leq 0.05$), and VO_{2PEAK} capacity (1.4 ± 0.2 L/min pretraining vs. 1.7 ± 0.3 L/min posttraining; $p \leq 0.05$). Though not significant, single-leg training also increased mean insulin resistance scores (CLIX-IR) by ~ 40% (3.3 ± 1.2 vs. 4.7 ± 2.9; $p = 0.15$). Our study demonstrates that HIIT can drive significant improvement in clinically relevant health measures such as HbA1c and VO_{2PEAK} capacity

in those at high risk for developing T2DM. Additionally, single-leg cycling is unique because it can minimize central hemodynamic stress (average heart-rate of 142 ± 15 beats/min at ≥ 80% of VO_{2PEAK}) while still providing adequate peripheral stimulus to drive adaptation. This is of great benefit when re-introducing a sedentary population to exercise.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to sincerely thank my committee members, James C. Martin, Russell S. Richardson, and Timothy E. Graham for their help and guidance during this project. I would like to acknowledge the Utah Vascular Research Laboratory, Neuromuscular Function Laboratory, and the University of Utah Center for Clinical and Translational Science for all their assistance in data collection. Finally, and most importantly, I'd like to thank my parents, Mark and Cheryl Wehmanen, for their unwavering support throughout my academic career.

INTRODUCTION

In 2012 more than 27 million Americans were suffering from Type II Diabetes (T2DM), which cost the U.S. $176 billion in direct health care costs, and $245 billion in total (Centers for Disease Control and Prevention, 2014). These costs result from the fact that diabetes is a burden to manage, and blood glucose levels need to be constantly monitored. Poorly controlled T2DM can result in severe health complications including vision loss, kidney failure, and lower limb amputation. Additionally, T2DM greatly increases the risk of heart disease and stroke, and individuals diagnosed with T2DM die from these diseases at a rate 2-4 times greater than nondiabetics (Centers for Disease Control and Prevention, 2011). Fortunately, lifestyle changes and exercise can improve the outlook for T2DM, and prevent those at risk of developing the disease from progressing. In T2DM pathology, skeletal muscle insulin resistance is a key early defect that contributes to the development of the disease (Henriksen, Diamond-Stanic, & Marchionne, 2011). As a result, defects in skeletal muscle glucose utilization, and exercise driven adaptations mitigating these defects are an important area of investigation.

Purpose Statement

The purpose of this study was to determine the effect of single-leg cycle training on parameters of insulin resistance and hyperglycemia.

Research Questions

Specifically, we investigated the effect of 6-weeks of high-intensity, interval based, single-leg cycle training on:

1. Insulin sensitivity (CLIX-IR)
2. Pancreatic beta-cell function (CLIX-Beta Cell)
3. Blood glucose levels (fasting blood glucose and HbA1c)

in individuals who displayed metabolic syndrome, insulin resistance, and subclinical hyperglycemia.

Research Hypotheses

In response to the stated research questions, we hypothesized that 6 weeks of high-intensity single-leg training would improve insulin sensitivity (CLIX-IR) and blood glucose levels (fasting blood glucose and HbA1c) in the specified individual.

REVIEW OF LITERATURE

The GLUT-4 Transporter and T2DM

A key step in glucose utilization is the transport of glucose from the blood into the cells for energy production. Previous studies (Cline et al., 1999; Morino, Petersen, & Shulman, 2006; Shepherd & Kahn, 1999) have shown that insulin-stimulated glucose uptake into skeletal muscle via glucose tranporter-4 (GLUT4) proteins is the rate-limiting step of glucose metabolism in both healthy and diabetic populations. Decreased response to insulin-stimulated glucose transport in skeletal muscle is one major component that contributes to hyperglycemia and insulin resistance in T2DM (Cline et al., 1999; Henriksen, 2002; Shepherd & Kahn, 1999). The impairment of glucose uptake in T2DM is multifaceted with sites of dysfunction in the insulin signal cascade (Shepherd & Kahn, 1999) as well as possible decreases in total GLUT4 density (Gaster et al., 2001). In several reports with human subjects, impaired insulin-stimulated glucose uptake seen in T2DM was not associated with decreased total muscle GLUT4 protein content (Schalin-Jäntti et al., 1994), but rather reduced GLUT4 translocation to the cell surface (Ryder et al., 2000). In fact, Ryder and colleagues (2000) reported that basal levels of cell surface GLUT4 were similar between age, weight, and VO2 matched control and T2DM subjects, but administration of insulin had a blunted response in T2DM, resulting in 71% less insulin-stimulated cell surface GLUT-4. In contrast, other investigators report that total GLUT4 density in slow-twitch insulin sensitive muscle fibers was reduced by 18%

in T2DM subjects (Gaster et al., 2001). More recently, Kampmann and colleagues (2011) reported that total GLUT4 expression was reduced by 30% in skeletal muscle from T2DM patients requiring insulin treatment when compared with either T2DM patients treated with oral anti-diabetics, or healthy controls. Furthermore, fasting plasma glucose and total GLUT4 density in slow-twitch muscle fibers were negatively correlated (r^2 = -0.49) in T2DM (Gaster et al., 2001), suggesting a link between total GLUT4 content and hyperglycemia. Additionally, Doehner and researchers (2010) reported that lower GLUT4 protein content significantly predicted impaired insulin sensitivity in patients suffering from chronic heart failure. The defect in T2DM glucose transport is likely due to both decreased total GLUT4 density and impaired insulin signaling/GLUT4 translocation. The end result remains decreased glucose clearance as demonstrated by Cline et al. (1999), who showed that T2DM patients displayed a rate of glucose uptake ~ 80% lower than healthy controls when plasma concentrations of both insulin and glucose were held constant. As will be discussed, simple interventions such as exercise, aimed at improving GLUT4 density, translocation, and function, can be valuable in the treatment and prevention of T2DM.

Mitochondrial Dysfunction and T2DM

Compounding the problem of decreased glucose uptake in T2DM is evidence that once glucose has entered the cell, mitochondrial function and substrate oxidation are also impaired. In support, Schrauwen-Hinderling and investigators (2007) used noninvasive magnetic resonance spectroscopy to show that in vivo muscle mitochondrial function was significantly decreased in T2DM. It is also generally accepted that skeletal muscle PGC-

1α, a positive regulator of mitochondrial biogenesis and gene expression, is consistently down regulated in T2DM as well (Lira et al., 2010). Also, decreased mitochondrial-biogenesis has been implicated in contributing to the pathogenesis of insulin resistance in skeletal muscle (Joseph et al., 2012). Patti (2004) reported that expression of both PGC-1α and PGC-1β were reduced in Mexican American T2DM subjects by up to 46%. In support, Kelley and colleagues (2002) reported that total mitochondrial area was reduced by 35% in patients with T2DM, while Morino et al. (2005) reported that mitochondrial density was reduced by 38% in the insulin-resistant offspring of T2DM patients. Additionally, citrate synthase (CS), an enzyme used as a measure of mitochondrial activity, was reduced by ~35% in T2DM individuals (Kelley et al., 2002). More recently, Ritov and researchers (2010) reported that there was no significant difference in total mitochondrial content or CS activity between middle-aged, sedentary, lean subjects and T2DM individuals. The average activity of the electron transport chain (ETC), however, was significantly decreased by ~40% in the T2DM patients (Kelley et al., 2002; Ritov et al., 2010). Similarly, Petersen et al. (2004) showed a 30% decrease in rate of mitochondrial ATP production in young, lean, insulin-resistant children of T2DM parents when compared with age, weight, and activity matched insulin-sensitive controls. The fact remains that mitochondrial function is impaired in T2DM, and is likely due to decreased total mitochondrial density and impaired ETC capacity. Fortunately, as will again be demonstrated, exercise provides a potent positive stimulus to halt or reverse mitochondrial impairments, and help improve the outlook of T2DM and insulin resistance.

Exercise and T2DM

Exercise has been shown time and again to be an effective stimulus for improving glucose transport, mitochondrial biogenesis, and insulin sensitivity. Bruce and colleagues (2004) reported that 8-weeks (24 total sessions) of combined endurance and high-intensity exercise training improved insulin sensitivity by ~30% in T2DM patients. Similarly, 10 to 12-weeks (48-60 total sessions) of endurance training improved oral glucose tolerance in insulin resistant subjects (Hughes et al., 1993) and whole body insulin-mediated glucose disposal in T2DM patients (Dela et al., 1995). Additionally, 2-weeks (6 total sessions) of high intensity interval training (HIIT) improved insulin sensitivity by 35% in sedentary adults (Hood et al., 2011). In a review by Henriksen (2002), rat models indicated that the exercise-induced improvement in insulin action on skeletal muscle glucose uptake was associated with both increased GLUT4 density and adaptation of mitochondrial enzymes for glucose oxidation. In humans, 20 sessions of mixed endurance and intermittent high-intensity exercise increased skeletal muscle GLUT4 expression by 20% (Hussey et al., 2011). Similarly, 24 endurance-training sessions increased GLUT4 expression by 22% in T2DM patients and 38% in obese subjects (Christ-Roberts et al., 2004), while 48 exercise bouts increased GLUT4 by 60% (Hughes et al., 1993). Perhaps most surprisingly, two different studies utilizing 6 total sessions of HIIT increased total GLUT4 protein content by ~260% in sedentary adults (Hood et al., 2011) and ~369% in T2DM patients (Little et al., 2011), suggesting that certain exercise paradigms are more efficacious than others in driving adaptation in T2DM.

With respect to mitochondrial function, exercise has also been shown to increase PGC-1α, CS, and cytochrome c oxidase IV (COX-IV), a rate-limiting protein of the ETC and oxidative capacity. In T2DM subjects, 2-weeks (6 total sessions) of HIIT was sufficient to significantly improve skeletal muscle COX-IV protein levels (Little et al., 2011), while 4-weeks (20 total sessions) of endurance training increased COX-IV expression by 49% (Hussey et al., 2011). Six sessions of HIIT in sedentary adults increased CS content by 31%, COX-IV content by 39%, and PGC-1α content by 56% (Hood et al, 2011), while 18 sessions of HIIT increased maximal activity of both COX-IV and CS by ~50% in healthy females (Talanian et al, 2010). Improving both GLUT4 function and mitochondrial health have implications in the treatment and prevention of insulin resistance and T2DM. Exercise is a proven stimulus to invoke these changes, but the most effective exercise modality remains unanswered (Little et al., 2011).

Rationale for Single-Leg Cycling

Recently, Abbiss and colleagues (2011) reported that high-intensity single-leg cycle training significantly increased skeletal muscle GLUT4 and ETC enzyme content, when compared with more traditional double-leg training. In fact, single-leg cycling increased total GLUT4 content by ~100% when compared to traditional double-leg cycling. Similarly, single-leg training increased the ETC intermediates cytochrome oxidase II (COX II) by ~100%, and COX IV by ~200% (Abbiss et al., 2011). Even more impressive was that these results were seen after only 6 exercise bouts in a previously trained population where it had been expected that adaptations were already near maximum. Additionally, despite higher individual leg workloads achieved during single-

leg cycling (198 W single-leg vs. 172 W double-leg), participants reported lower ratings of perceived exertion (RPE; 16 single-leg vs. 18 double-leg) and similar perceptions of quadriceps pain (Abbiss, 2011). Similarly, both average and peak heart rates were significantly lower during single-leg cycling (145 beats/min single-leg vs. 164 beats/min double-leg, and 168 beats/min single-leg vs. 180 beats/min double-leg; Abbiss, 2011). These results, respective to exercise exertion and single-leg cycling, provide a unique training paradigm for individuals with insulin resistance, metabolic syndrome, or T2DM. Many of these individuals lead a sedentary lifestyle and display a low VO2 capacity. Asking them to step into a rigorous, whole-body, large muscle-mass training regimen would prove difficult. Single-leg cycling presents a unique paradigm where a smaller muscle mass can be trained at relatively high intensity while not over-taxing the participant's central hemodynamic capacity. Additionally, reports of lower RPE from single-leg training may improve exercise program adherence for previously sedentary individuals. We believe that if single-leg cycling can increase proteins and enzymes of glucose utilization (GLUT4, COX II and IV) so dramatically in elite athletes, then it may drive even larger adaptations in previously untrained, insulin resistant individuals, resulting in improved health.

METHODS

Recruitment

Individuals with insulin resistance and metabolic syndrome, but without frank T2DM or other complications were recruited for the current study. Prior to any study procedures, participants were informed about the protocols and potential risks both verbally and in writing. Informed written consent was obtained from all the participants prior to data collection. All study protocols were reviewed and were in accordance with the Institutional Review Boards of the University of Utah and Veterans Affairs Salt Lake City Health Care System. Volunteers who displayed a waist circumference of > 40 inches in men, or > 35 inches in women, were recruited from flyers placed at the University of Utah Hospital and School of Medicine. Initial screening for the presence of metabolic syndrome was conducted at PEAK Health and Fitness at the University of Utah. The presence of metabolic syndrome was confirmed if the volunteer met the previous criteria for waist circumference and displayed any two of the following measures: fasting blood glucose > 100 mg/dL, total fasting blood lipids > 150 mg/dL, HDL cholesterol < 50 mg/dL in women or < 40 mg/dL in men, or blood pressure > 130/85 mm Hg. Upon confirmation of metabolic syndrome, participants received a secondary screening for insulin resistance at the University of Utah Center for Clinical and Translational Science (CCTS). To determine if volunteers were insulin resistant, a five-time-point oral glucose tolerance test (OGTT) was administered, and blood glucose, insulin, and C-peptide levels

were monitored over the course of 2 hours. Severity of insulin resistance pre- and posttraining was determined using the Clamp Like Index Model (CLIX; Anderwald et al., 2007). In humans, the severity of hyperglycemia is determined by a combination of pancreatic β-cell dysfunction and peripheral insulin resistance. CLIX is mathematical model that utilizes the five-time-point OGTT (0, 30, 60, 90, 120 minute) area under the curve (AUC) for plasma glucose and C-peptide levels to estimate peripheral insulin resistance (CLIX-IR) and pancreatic β-cell function (CLIX-Beta Cell; Anderwald et al., 2007). Participants were considered insulin resistant if their CLIX-IR scores were < 6.0. Any participant whose OGTT indicated frank T2DM was referred directly to a physician for proper care. Additionally, any participant with symptoms of clinically relevant conditions that contraindicated intense exercise was also excluded.

Design

A comparison of pre- and posttraining measures was used to determine the effect of a 6-week (12 session) exercise program on a number of health and metabolic parameters. Specifically, measures taken before and after high-intensity single-leg cycle training were used to assess changes in insulin resistance (CLIX-IR), pancreatic beta-cell function (CLIX-beta-cell), and blood glucose levels (fasting plasma glucose, HbA1c), as well as total blood lipids, HDL and LDL cholesterol, and VO2PEAK capacity.

Oral Glucose Tolerance Test

Prior to training and again 14-48 hrs after their final exercise session, participants reported to the University of Utah CCTS for an OGTT. Participants reported to the CCTS

between 7:00 – 8:00AM following an overnight fast (12 hrs). Upon admittance an IV was placed into the individual's antecubital vein, using an intracatheter needle (20–22 gauge). The intracatheter needle was kept patent between samples with a slow saline drip using 3-way stopcock or saline flush after each blood draw. Baseline blood samples were drawn at the time of the IV placement. Following the draw of baseline samples, the patient was given a standard 75g glucose load. The entire solution was consumed within 5 minutes, and a timer was started at the "0 minute" when the patient finished their glucose drink. Subsequent blood samples were then drawn at the 30, 60, 90, and 120-minute time points. All samples were centrifuged on site, refrigerated, and then shipped overnight to Human Diagnostic Laboratories (Richmond, VA) to be analyzed for glucose, C-peptide, and insulin levels. Additionally, a comprehensive panel of baseline metabolic parameters were also measured and analyzed.

Training

Prior to the training sessions, participants completed a single-leg VO2PEAK test to establish a baseline training intensity. Throughout the VO2PEAK test, gas exchange was assessed using indirect calorimetry on a TrueOne 2400 metabolic system (Parvo Medics, Sandy UT). Before each test, both the gas analyzer and ventilometer were calibrated. Participants were given 5 minutes of self-selected warm-up before beginning the VO2PEAK protocol. During the single-leg VO2PEAK test, participants began cycling at 20W, with power increasing 12.5W each minute thereafter. Termination of the protocol occurred when any two of the following criteria were met: the participant's RER had risen above 1.1, they had reached their age predicted maximum HR, they reported a

rating of perceived exertion (RPE) (Borg's 6-20 scale) of 19 or above, or they were unable to maintain a pedaling cadence of > 60rpm. VO2PEAK power was determined from the highest power stage completed in full.

All participants then completed a minimum of 12 sessions of high-intensity single-leg cycle training over a period of ~40 days. All training was conducted on modified Monark cycle ergometers (HealthCare International Inc., Langley, WA) equipped with SRM Powermeters (SRM Service Center Inc., Colorado Springs, CO) under supervision at the Cardiac Rehabilitation Gym in the University of Utah Hospital. To preserve traditional cycling biomechanics during single-leg cycle training, a counterweight was fitted to the contra-lateral crank arm of each ergometer (Abbiss et al., 2011; Thomas & Martin, 2009).

Each single-leg training session required the completion of three intervals, 4 minutes in length, on each leg (24 total minutes of training). The 4 minute training intervals were separated by 6 minutes of active recovery. During all training intervals, participants were provided with continuous feedback of power output. Target training power was initially set at 80% of VO2PEAK power. Target training powers were then increased 4% per week for the following 5 weeks (Hickson, Bonzes, & Holloszy, 1977). This resulted in a total power increase of 20% over the course of the training regimen. Additionally, participants were encouraged to give their best effort, which in some cases exceeded the target power. The initial leg each participant used during single-leg cycling (right vs. left) was counterbalanced.

Heart rate (HR; Polar, Polar Electro Inc., Lake Success NY) was continuously recorded during all training sessions. Ratings of perceived exertion (RPE; Borg's 6-20

scale) for the whole body and leg-only were recorded at the 4 minute mark of each interval. Total work completed during single-leg intervals was calculated using the following formula: work (J)= P X t, where P was the average power output (W) produced during the interval and t was the total time (s) performing intervals. Total work completed during single-leg cycling was calculated as the sum of the work from both right and left legs.

Statistical Analyses

Separate one-way repeated measures ANOVAs (pretraining vs. posttraining) were used to determine if there were any changes in insulin resistance (CLIX-IR), pancreatic beta-cell function (CLIX-Beta cell), fasting blood glucose, HbA1c, VO2PEAK capacity, and blood lipids following training. Significance for all comparisons was set at $P \leq 0.05$. All values reported in text and graphically are means ± standard deviation.

RESULTS

Training

Seven participants with insulin resistance and metabolic syndrome completed a minimum of 12 high-intensity single-leg training sessions. Physical and health characteristics are reported in Table 4.1. Three participants completed additional training sessions to accommodate posttest scheduling. Two participants completed 14 total training visits, while a third individual completed one extra training session, for a total of 13. Average power output during all single-leg training intervals was 77 ± 13 W. During the 1st week of training, average power output was 65 ± 15 W, while average power output during the 6th week of training was 85 ± 12 W, resulting in a 31% ($p \leq 0.01$) increase in training power. Average work completed over the entire training regimen was 1405 ± 264 kJ. The average HR for all participants throughout training was 142 ± 15 beats/min, while mean peak HR was 154 ± 17 beats/min. During the final 10 seconds of each interval, mean reported whole-body RPE was 15 ± 2, while RPE of the exercising leg was 17 ± 2. Training data are summarized in Table 4.2.

Performance

Prior to training, absolute single-leg VO2PEAK capacity was 1.4 ± 0.2 L/min, and maximal aerobic power was 73 ± 17 W. After training, absolute VO2PEAK capacity

increased by 19.5% ($p \leq 0.05$) to 1.7 ± 0.3 L/min (Figure 4.1), while maximal aerobic power increased by 39% to 102 ± 20 W ($p \leq 0.01$).

Insulin Resistance and Glucose Control

Single-leg training significantly improved chronic blood glucose levels, assessed via HbA1c, from 5.8 ± 0.5% pretraining to 5.6 ± 0.4% posttraining ($p \leq 0.05$; Figure 4.2). Though not statistically significant, mean CLIX-IR scores increased by 41% following training (3.3 ± 1.2 vs. 4.7 ± 2.9; $p = 0.15$), while pancreatic beta-cell function, estimated via CLIX-beta cell, increased by 59% (3.1 ± 1.3 vs. 4.9 ± 4.2; $p = 0.25$; Figure 4.3). Fasting insulin (18.9 ± 14.8 μU/mL pretraining vs. 23.1 ± 24.2 μU/mL posttraining; $p = 0.49$), pancreatic C-peptide (3.6 ± 1.4 ng/mL pretraining vs. 3.5 ± 1.9 ng/mL posttraining; $p = 0.74$), and fasting plasma glucose levels (99.9 ± 20.4 mg/dL pretraining vs. 97.3 ± 12.9 mg/dL posttraining; $p = 0.48$) were all unchanged following training. Finally, though again not significant, training decreased 2 hour OGTT blood glucose and insulin levels. Two-hour OGTT blood glucose decreased 23% (155 ± 48 mg/dL vs. 120 ± 27 mg/dL; $p = 0.16$), while 2 hour insulin levels decreased 33% (111 ± 56 μU/mL vs. 74 ± 58 μU/mL; $p = 0.21$; Figure 4.4).

Health Parameters

Both weight (103.6 ± 17.7 kg pretraining vs. 104.1 ± 17.4 kg posttraining; $p = 0.45$) and BMI (38 ± 6 kg/m2 pretraining vs. 38 ± 6 kg/m2 posttraining; $p = 0.40$) were unchanged following training. Because of incomplete lab reports, changes in blood lipid levels were assessed in 5 of the 7 participants. Training did not induce changes in the

concentration of high-density lipoprotein cholesterol (HDL; 54.2 ± 17.3 mg/dL pretraining vs. 52.4 ± 13.4 mg/dL posttraining; $p = 0.64$), low-density lipoprotein cholesterol (LDL; 42.2 ± 17.5 mg/dL pretraining vs. 42.6 ± 18.8 mg/dL posttraining; $p = 0.93$), or total triglyceride levels (170.6 ± 79.0 mg/dL pretraining vs. 167.4 ± 64.8 mg/dL posttraining; $p = 0.88$).

Table 4.1:
Clinical and anthropometric characteristics of all participants.

	N = 7
Age (yrs)	43 ± 14
Height (cm)	165.0 ± 11.6
Weight (kg)	103.6 ± 17.7
BMI (kg/m2)	38.1 ± 5.6
CLIX-IR	3.3 ± 1.2
CLIX-Beta Cell	3.1 ± 1.3
HbA1c (%)	5.8 ± 0.5
Fasting Blood Glucose (mg/dL)	99.9 ± 20.4
Total Triglycerides (mg/dL)	171.3 ± 70.6
HDL-Cholesterol (mg/dL)	51.7 ± 16.7
LDL-Cholesterol (mg/dL)	41.0 ± 15.9
Single-leg VO2 Peak (L/min)	1.4 ± 0.2
Relative VO2 Peak (mL/kg/min)	14.0 ± 3.8
VO2 Peak Power (W)	73 ± 17
Values	Mean ± S.D.

Table 4.2:
Summary of training data.

Average Power (W)	Average HR (beats/min)	Peak HR (beats/min)	RPE Whole Body	RPE Leg Only	Total Work (kJ)
77 ± 13	142 ± 15	154 ± 17	15 ± 2	17 ± 2	1405 ± 264
All Values are Means ± S.D.					

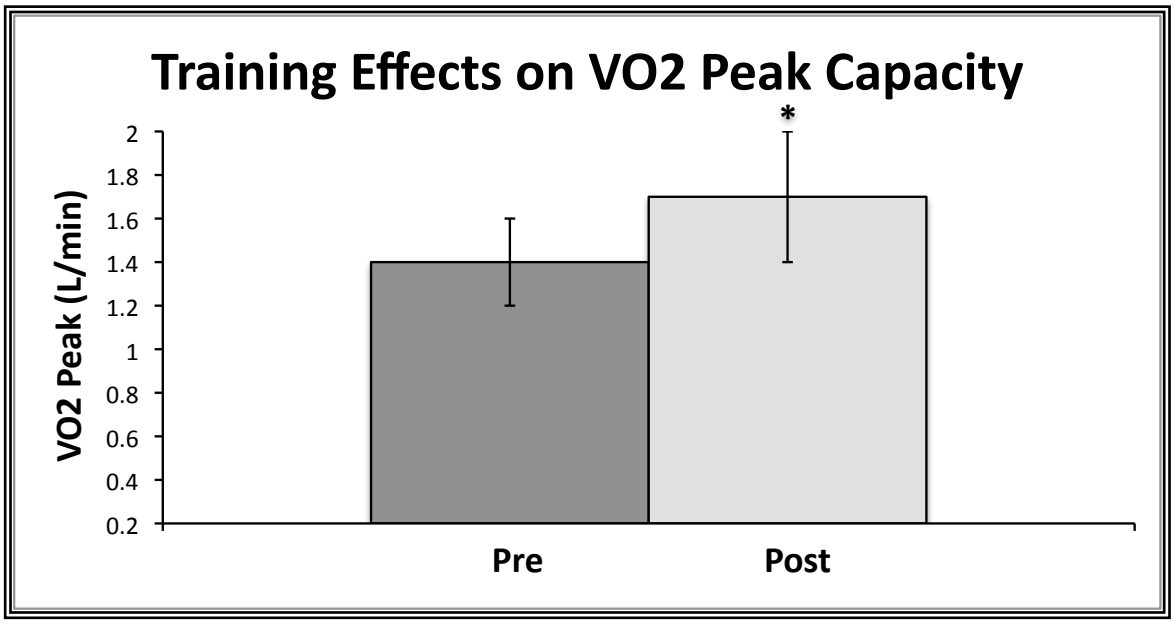

Figure 4.1: Training effects on VO_{2PEAK} capacity. Training increased absolute single-leg VO2PEAK capacity by 19.5% from 1.4 ± 0.2 L/min to 1.7 ± 0.3 L/min (*$p \leq 0.05$)

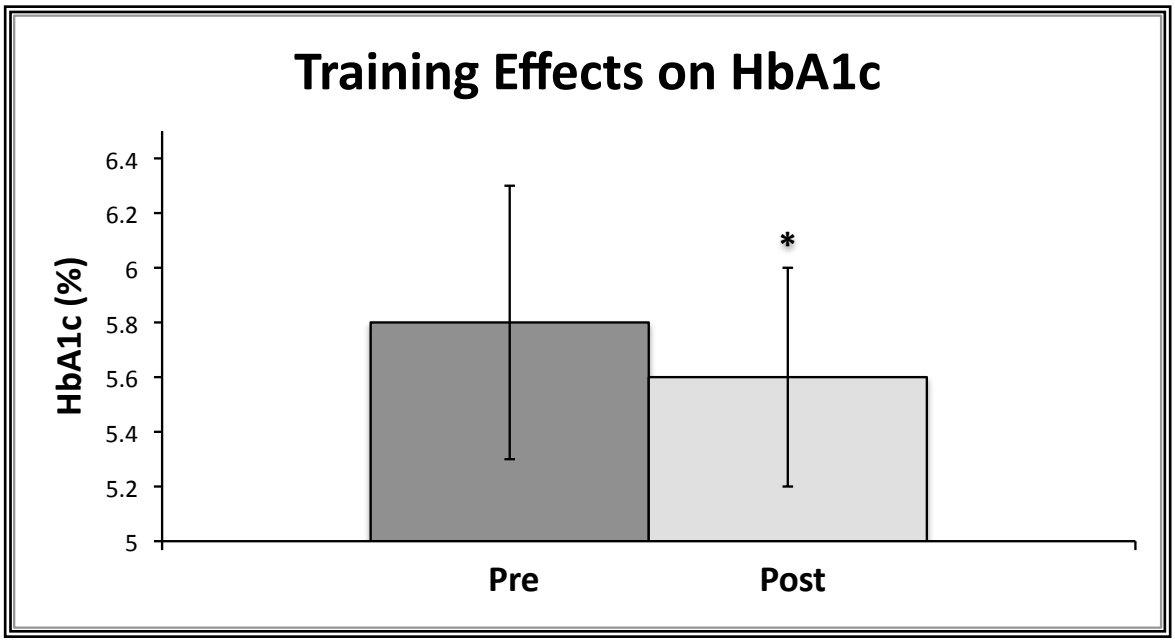

Figure 4.2: Training effects on HbA1c. Chronic blood glucose levels (HbA1c) were decreased 3.5% following training from prediabetic level (5.8 ± 0.5%) to within the normal range (5.6 ± 0.4%; *$p \leq 0.05$).

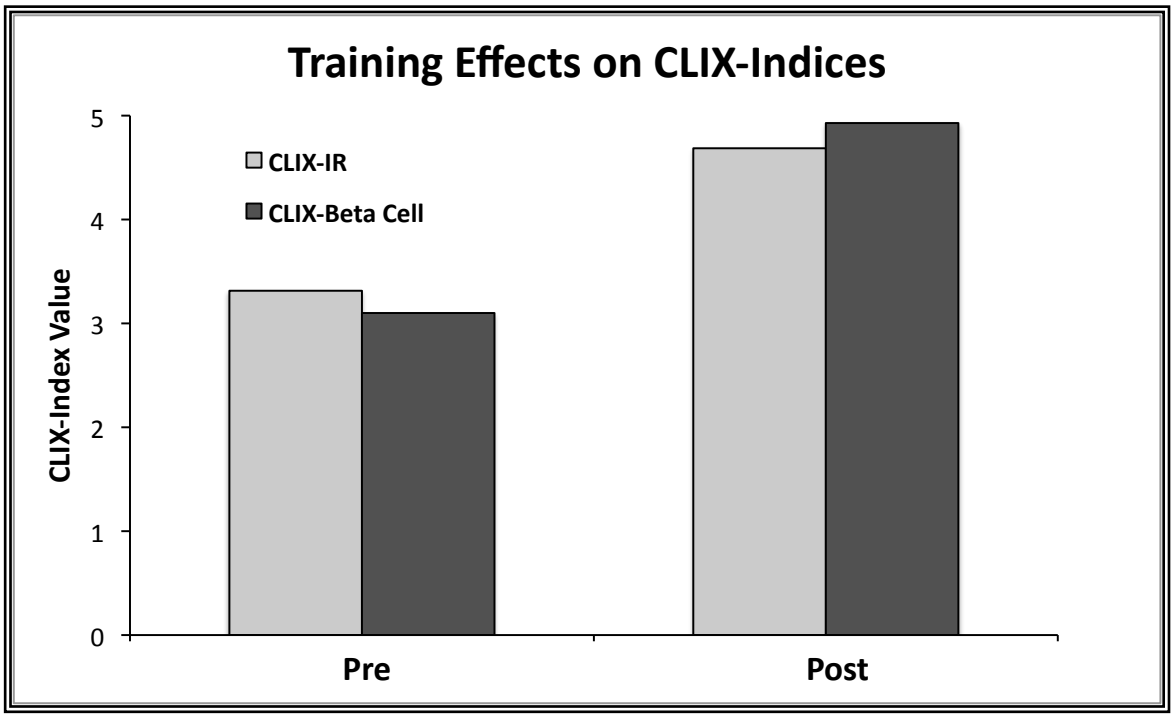

Figure 4.3: Training effects on CLIX-Indices. Though not statistically significant, insulin sensitivity (CLIX-IR) and pancreatic beta-cell function (CLIX-Beta Cell) were increased following training. Average CLIX-IR scores were increased by 41% (3.3 ± 1.2 vs. 4.7 ± 2.9; $p = 0.15$), while CLIX-beta cell scores increased by 59% (3.1 ± 1.3 vs. 4.9 ± 4.2; $p = 0.25$).

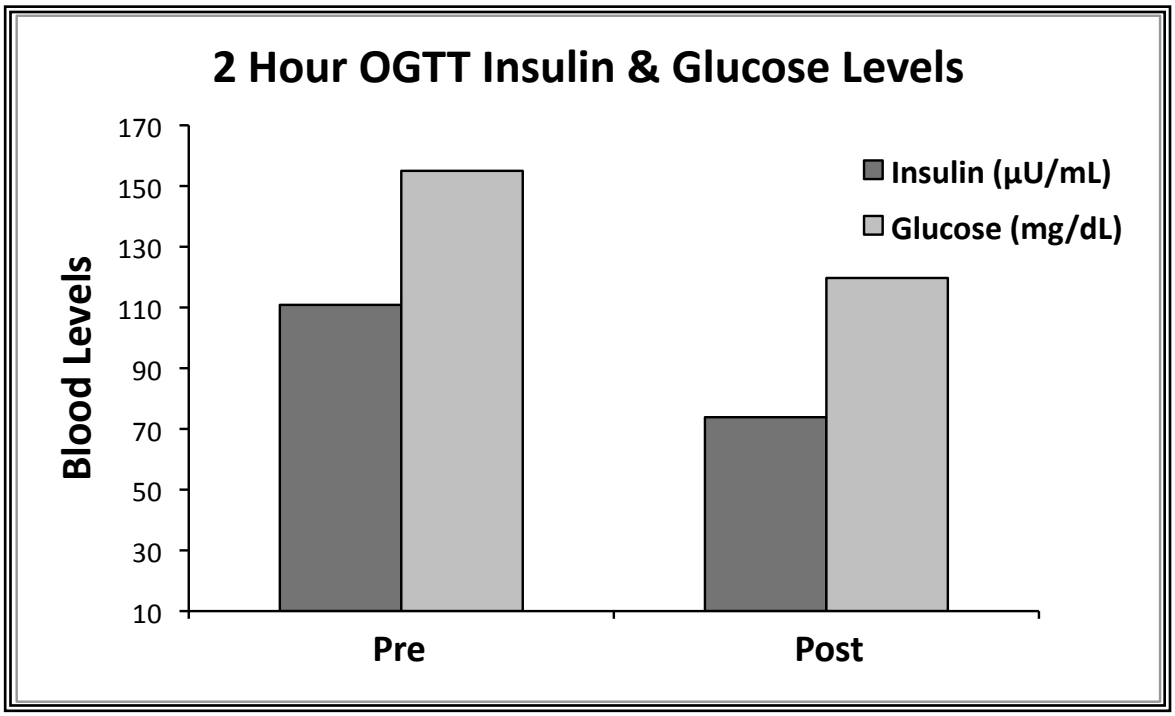

Figure 4.4: Two hour OGTT glucose and insulin. Though not statistically significant, 2 hour OGTT blood glucose and insulin levels were decreased following training. Average 2 hour blood glucose levels were decreased by 23% (155 ± 48 mg/dL vs. 120 ± 27 mg/dL; $p = 0.16$), while 2 hour insulin levels were decreased by 33% (111 ± 56 µU/mL vs. 74 ± 58 µU/mL; $p = 0.21$).

DISCUSSION AND CONCLUSION

Our purpose was to investigate the effect of high-intensity, interval based, single-leg training on insulin resistance, pancreatic beta-cell function, blood glucose levels, and exercise capacity in individuals with insulin resistance and metabolic syndrome. Our principal finding was that 12-14 sessions of single-leg HIIT significantly improved HbA1c, a clinically relevant measure of chronic blood glucose levels. The importance of HbA1c levels has been previously documented. Adams and colleagues (2009) reported that elevated HbA1c levels were significantly associated with the macrovascular diseases, cardiovascular disease (CVD), coronary heart disease (CHD), and stroke in nondiabetics. This relationship was established in both men and women after adjustment for factors including BMI and waist circumference (Adams et al., 2009). Similarly, Borg et al. (2011) showed that among an array of blood glucose indicators, HbA1c had the strongest association with CVD risk in individuals with T2DM. A review by Zanuso (2010) supports exercise as a way to decrease HbA1c and improve glucose control. Specifically, 6 months of endurance exercise was shown to decrease average HbA1c levels by 4% in obese T2DM patients (Hansen, 2009). In our current study, we found that 12-14 sessions of single-leg cycling improved HbA1c levels by 3.5% ($p \leq 0.05$) in obese insulin-resistant individuals. Thus, our intervention elicited similar improvements to those shown by Hansen (2009), but in one-quarter the time. Additionally, our reported improvement decreased HbA1c from 5.8%, which is classified as prediabetic, to 5.6%, which is within

the normal range. The finding that single-leg cycle training can improve HbA1c levels from the prediabetic range to normal is impressive, and strongly supports high-intensity, targeted training for those with chronically elevated blood glucose. Moreover, high-intensity single-leg training elicited these changes rapidly thus minimizing exposure to the risks associated with elevated HbA1c.

In addition to improving chronic blood glucose levels, 6 weeks of single-leg training significantly improved cardio-respiratory fitness (CRF). This is also of great importance because of the effect aerobic capacity has on overall health. A study from the Aerobics Center Longitudinal Study (ACLS) compared all-cause and CVD mortality across fitness levels. When looking at the sexes independently, they found that the most fit men had a 43% lower risk for all-cause mortality and a 47% lower risk of CVD mortality, while the most fit women had 53% lower risk of all-cause mortality and 70% lower risk of CVD mortality (Lee et al., 2010). Similarly, in men with metabolic syndrome, those with the lowest CRF had a 2.25 greater risk of CVD mortality than those who were most fit (Katzmarzyk, Church, & Blair, 2004). A prospective study by Erikssen et al. (1998) followed ~2000 healthy men and reported that improvements in CRF over a 7 year period were associated with a significantly lower risk of all-cause mortality for an additional 15 years of follow-up. Moreover, this protective effect was found to be independent of fitness level at baseline, or changes in body weight across the follow-up period (Erikssen, 1998). With respect to exercise, 48 sessions of aerobic training at 50% of maximal HR reserve increased aerobic capacity of double-leg cycling by 8.2% (Hughes et al., 1993). In comparison, Davies and Sargeant (1975) reported that 18 sessions of single-leg exercise, 60 minutes in length, improved single-leg VO2PEAK

capacity by 14.0%, which corresponded to a 4.6% increase in double-leg VO2PEAK. In our study, 12-14 exercise sessions, incorporating only 24 minutes of high intensity exercise, improved single-leg VO2PEAK by 19.5%. This dramatic increase in CRF over a short time period provides further support that high-intensity, targeted training can improve overall health in those with insulin resistance, elevated blood glucose, and metabolic syndrome. The fact that improving aerobic capacity also decreases the risk of death from CVD is of utmost importance for individuals with elevated blood glucose levels, as these people are at higher risk for macrovascular diseases to begin with.

To assess insulin resistance we used the CLIX-IR model, which utilizes the 5-time-point-OGTT AUC for plasma glucose and C-peptide levels to estimate glucose infusion rates (GIR; Anderwald et al., 2007). CLIX-IR was developed to accurately model the current "gold-standard" of measuring insulin sensitivity, the hyperinsulinemic clamp test, and is currently the most accurate way to assess insulin sensitivity from an easily performed OGTT. Improvements in insulin resistance following 6 weeks of single-leg cycling were highly variable. Two of 7 participants showed no response, while the remaining 5 all improved, and in one instance, a ~112% increase in CLIX-IR was measured. In total, 6 weeks of single-leg training increased CLIX-IR scores by ~40%, although results were not statistically significant. Exercise is proven stimulus to improve insulin action, but the most effective training remains unproven. Forty-eight sessions of aerobic exercise was reported to improve insulin-stimulated glucose disposal (GIR) by 11% (Hughes et al., 1993). More recently, both Hood et al. (2011) and Bruce and colleagues (2004) reported that 6-24 sessions of exercise incorporating HIIT was sufficient to improve insulin sensitivity by at least 30% in both sedentary adults and

T2DM patients. Again, while not statistically significant, our mean response to changes in insulin resistance were even greater than these, and when considered alongside the Hood (2011) and Bruce (2004) studies, HIIT training appears to be a very potent stimulus for improving insulin action.

Additionally, the single-leg cycling model is unique in that the exercising muscle can be worked at high intensity, >= 80% of VO2PEAK capacity, while central hemodynamic stress remains relatively low. Average heart rate for all participants during the 4 minute high-intensity single-leg intervals was 142 beats/min, while average ratings of perceived exertion, recorded using Borg's 6-20 RPE scale, were 15 whole-body and 17 for the exercising leg. Our average RPE values of 15 (defined as "hard") and 17 ("very hard") reinforce single-leg cycling's ability to highly stress the exercising muscle while moderating central hemodynamic load. This is of great benefit to obese, insulin resistant, and prediabetic populations who likely have low CRF. In many cases, these individuals may be so limited by low aerobic capacity that traditional exercise would not allow them to work at a level sufficient to drive meaningful adaptation of exercising muscle. Four months of low intensity exercise, consisting of 150 minutes of brisk walking per week, did not change HbA1c levels or CRF in T2DM individuals (Fritz et al., 2006). Single-leg cycling is unique because it can provide a large stimulus to drive peripheral adaptation over a short time period (12-14 sessions), while simultaneously minimizing central hemodynamic stress and perceived effort to a level that allows previously sedentary individuals to see quick improvements in health and adhere to an exercise program.

Limitations

Limitations to the current study included difficulty locating and identifying qualified participants, resulting in a small cohort. An initial goal of our study design was to implement single-leg training in a drug naïve population. Finding study participants who exhibited insulin resistance, and subclinical hyperglycemia but who were not frank diabetic or on blood glucose medication proved difficult. A larger cohort size would have increased study power and helped definitively answer several of our research questions, specifically, what impact high-intensity single-leg training had on insulin resistance (CLIX-IR). Additionally, limited flexibility in exercise times was an issue. Safety concerns required that all exercise for the current study take place in a hospital setting. As a result, training availability was limited to twice a week, Tuesday and Thursday. This resulted in 4 off days between every second exercise session. Ideally, training would have taken place 3 days a week limiting the number of off-days to 2 in a row, and presumably increasing adaptive stimulus.

Future Research

An important direction for future research is investigation into the effect of single-leg training on individuals already diagnosed with frank T2DM. Preventing individuals at risk of developing diabetes from progressing is important, but equally relevant is improving the health of those who have already been diagnosed. It is known that exercise is important to those suffering from T2DM, but further investigation of high-intensity single-leg exercise is warranted. Additionally, combining targeted HIIT with other lifestyle modifications may even prove to reverse T2DM in some individuals.

Summary

Twelve to 14 sessions of high-intensity, interval-based, single-leg training significantly improved chronic blood glucose levels and CRF in obese individuals with insulin resistance, and metabolic syndrome. These findings provide evidence that targeted, high-intensity, small muscle mass exercise can be utilized to drive significant improvement in clinically relevant health measures, while simultaneously minimizing central hemodynamic stress, and levels of perceived exertion. This is of benefit when considering the safety of re-introducing sedentary individuals to exercise, while also encouraging them to work at levels great enough to drive adaptation.

REFERENCES

Abbiss, C.R., Karagounis, L.G., Laursen, P.B., Peiffer, J.J., Martin, D.T., Hawley, J.A., & Martin, J.C. (2011). Single-leg cycle training is superior to double-leg cycling in improving the oxidative potential and metabolic profile of trained skeletal muscle. *Journal of Applied Physiology, 110*(5), 1248-1255.

Adams, R., Appleton, S., Hill, C., Wilson, D., Taylor, A., Chittleborough, C., & Ruffin, R. (2009). Independent association of HbA1c and incident cardiovascular disease in people without diabetes. *Obesity, 17*(3), 559-563.

American Diabetes Association. Economic costs of diabetes in the U.S. in 2007. *Diabetes Care, 31*(3), 576–615.

Anderwald, C., Anderwald-Stadler, M., Promintzer, M., Prager, G., Mandl, M., Nowotny, P., & Krebs, M. (2007). The Clamp-Like Index A novel and highly sensitive insulin sensitivity index to calculate hyperinsulinemic clamp glucose infusion rates from oral glucose tolerance tests in nondiabetic subjects. *Diabetes Care, 30*(9), 2374-2380.

Borg, R., Kuenen, J., Carstensen, B., Zheng, H., Nathan, D., Heine, R., & Witte, D. (2011). HbA1c and mean blood glucose show stronger associations with cardiovascular disease risk factors than do postprandial glycaemia or glucose variability in persons with diabetes: The A1C-Derived Average Glucose (ADAG) study. *Diabetologia, 54*(1), 69-72.

Bruce, C.R., Kriketos, A.D., Cooney, G.J., & Hawley, J.A. (2004). Disassociation of muscle triglyceride content and insulin sensitivity after exercise training in patients with type 2 diabetes. *Diabetologia, 47*(1), 23-30.

Camps, M., Castello, A., Munoz, P., Monfar, M., Testar, X., Palacin, M., & Zorzano, A. (1992). Effect of diabetes and fasting on GLUT-4 (muscle/fat) glucose-transporter expression in insulin-sensitive tissues. Heterogeneous response in heart, red and white muscle. *Biochemical Journal, 282*(3), 765.

Centers for Disease Control and Prevention. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. Atlanta, GA: U.S. Department of Health and Human Services; 2014.

Centers for Disease Control and Prevention. National Diabetes Fact Sheet, 2011. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services; 2011.

Christ-Roberts, C.Y., Pratipanawatr, T., Pratipanawatr, W., Berria, R., Belfort, R., Kashyap, S., & Mandarino, L.J. (2004). Exercise training increases glycogen synthase activity and GLUT4 expression but not insulin signaling in overweight nondiabetic and type 2 diabetic subjects. *Metabolism, 53*(9), 1233-1242.

Cline G.W., Falk-Petersen K., Krssak M., Shen J., Hundal R.S., Trajanoski Z., Inzucchi S., Dresner A., Rothman D.L., & Shulman G.I. (1999). Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *New England Journal of Medicine, 341*, 240–246.

Davies, C., & Sargeant, A. (1975). Effects of training on the physiological responses to one-and two-leg work. *Age, 55*, 1.

Dela, F., Larsen, J.J., Mikines, K.J., Ploug, T., Petersen, L.N., & Galbo, H. (1995). Insulin-stimulated muscle glucose clearance in patients with NIDDM: Effects of one-legged physical training. *Diabetes, 44*(9), 1010-1020.

Doehner, W., Gathercole, D., Cicoira, M., Krack, A., Coats, A. J., Camici, P.G., & Anker, S.D. (2010). Reduced glucose transporter GLUT4 in skeletal muscle predicts insulin resistance in non-diabetic chronic heart failure patients independently of body composition. *International Journal of Cardiology, 138*(1), 19-24.

Erikssen, G., Liestøl, K., Bjørnholt, J., Thaulow, E., Sandvik, L., & Erikssen, J. (1998). Changes in physical fitness and changes in mortality. *The Lancet, 352*(9130), 759-762.

Fritz, T., Krämer, D., Karlsson, H., Galuska, D., Engfeldt, P., Zierath, J., & Krook, A. (2006). Low□intensity exercise increases skeletal muscle protein expression of PPARδ and UCP3 in type 2 diabetic patients. *Diabetes/Metabolism Research and Reviews, 22*(6), 492-498.

Gaster, M., Staehr, P., Beck-Nielsen, H., Schrøder, H.D., & Handberg, A. (2001). GLUT4 is reduced in slow muscle fibers of type 2 diabetic patients: Is insulin resistance in type 2 diabetes a slow type 1 fiber disease? *Diabetes, 50*(6), 1324-1329.

Hansen, D., Dendale, P., Jonkers, R. A., Beelen, M., Manders, R., Corluy, L., & van Loon, L. (2009). Continuous low-to moderate-intensity exercise training is as effective as moderate-to high-intensity exercise training at lowering blood HbA1c in obese type 2 diabetes patients. *Diabetologia, 52*(9), 1789-1797.

Hennessey, J.V., Chromiak, J.A., Dellaventura, S., Guertin, J., & Maclean, D.B. (1997). Increase in percutaneous muscle biopsy yield with a suction-enhancement technique. *Journal of Applied Physiology, 82*(6), 1739-1742.

Henriksen, E.J. (2002). Invited review: Effects of acute exercise and exercise training on insulin resistance. *Journal of Applied Physiology, 93*(2), 788-796.

Henriksen, E.J., Diamond-Stanic, M.K., & Marchionne, E.M. (2011). Oxidative stress and the etiology of insulin resistance and type 2 diabetes. *Free Radical Biology and Medicine, 51*(5), 993-999.

Hickson, R.C., Bomze, H.A., & Holloszy, J.O. (1977). Linear increase in aerobic power induced by a strenuous program of endurance exercise. *Journal of Applied Physiology, 42*(3), 372-376.

Hood, M.S., Little, J.P., Tarnopolsky, M.A., Myslik, F., & Gibala, M.J. (2011). Low-volume interval training improves muscle oxidative capacity in sedentary adults. *Medicine and Science in Sports and Exercise, 43*(10), 1849-56.

Hughes, V.A., Fiatarone, M.A., Fielding, R.A., Kahn, B.B., Ferrara, C.M., Shepherd, P.E. & Evans, W.J. (1993). Exercise increases muscle GLUT-4 levels and insulin action in subjects with impaired glucose tolerance. *American Journal of Physiology-Endocrinology And Metabolism, 264*(6), E855-E862.

Hussey, S.E., McGee, S.L., Garnham, A., Wentworth, J.M., Jeukendrup, A. E., & Hargreaves, M. (2011). Exercise training increases adipose tissue GLUT4 expression in patients with type 2 diabetes. *Diabetes, Obesity and Metabolism, 13*(10), 959-962.

Joseph, A.M., Joanisse, D.R., Baillot, R.G., & Hood, D.A. (2011). Mitochondrial dysregulation in the pathogenesis of diabetes: Potential for mitochondrial biogenesis-mediated interventions. *Experimental Diabetes Research, 2012.*

Kampmann, U., Christensen, B., Nielsen, T.S., Pedersen, S. B., Ørskov, L., Lund, S., & Jessen, N. (2011). GLUT4 and UBC9 protein expression is reduced in muscle from type 2 diabetic patients with severe insulin resistance. *PloS One, 6*(11), e27854.

Katzmarzyk, P., Church, T., & Blair, S. (2004). Cardiorespiratory fitness attenuates the effects of the metabolic syndrome on all-cause and cardiovascular disease mortality in men. *Archives of Internal Medicine, 164*(10), 1092-1097.

Kelley, D.E., He, J., Menshikova, E.V., & Ritov, V.B. (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes, 51*(10), 2944-2950.

Lee, D., Artero, E, Sui, X., & Blair, S. (2010). Review: Mortality trends in the general population: The importance of cardiorespiratory fitness. *Journal of Psychopharmacology, 24*(4 suppl), 27-35.

Little, J.P., Gillen, J.B., Percival, M E., Safdar, A., Tarnopolsky, M.A., Punthakee, Z., & Gibala, M.J. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *Journal of Applied Physiology, 111*(6), 1554-1560.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., & Turner, R.C. (1985). Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28*(7), 412-419.

Morino, K., Petersen, K F., Dufour, S., Befroy, D., Frattini, J., Shatzkes, N., & Shulman, G.I. (2005). Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. *Journal of Clinical Investigation, 115*(12), 3587-3593.

Morino, K., Petersen, K.F., & Shulman, G.I. (2006). Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes, 55*(Supplement 2), S9-S15.

O'gorman, D.J., Karlsson, H.K.R., McQuaid, S., Yousif, O., Rahman, Y., Gasparro, D., & Nolan, J.J. (2006). Exercise training increases insulin-stimulated glucose disposal and GLUT4 (SLC2A4) protein content in patients with type 2 diabetes. *Diabetologia, 49*(12), 2983-2992.

Patti, M.E. (2004). Gene expression in humans with diabetes and prediabetes: What have we learned about diabetes pathophysiology? *Current Opinion in Clinical Nutrition & Metabolic Care, 7*(4), 383-390.

Petersen, K.F., Dufour, S., Befroy, D., Garcia, R., & Shulman, G.I. (2004). Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *New England Journal of Medicine, 350*(7), 664-671.

Ritov, V.B., Menshikova, E.V., Azuma, K., Wood, R., Toledo, F.G., Goodpaster, B.H., & Kelley, D.E. (2010). Deficiency of electron transport chain in human skeletal muscle mitochondria in type 2 diabetes mellitus and obesity. *American Journal of Physiology-Endocrinology And Metabolism, 298*(1), E49-E58.

Ritov, V.B., Menshikova, E.V., He, J., Ferrell, R.E., Goodpaster, B.H. & Kelley, D.E., (2005). Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. *Diabetes, 54*(1), 8–14.

Ryder, J.W., Yang, J., Galuska, D., Rincon, J., Björnholm, M., Krook, A., & Holman, G.D. (2000). Use of a novel impermeable biotinylated photolabeling reagent to assess insulin-and hypoxia-stimulated cell surface GLUT4 content in skeletal muscle from type 2 diabetic patients. *Diabetes, 49*(4), 647-654.

Schalin-Jäntti, C., Yki-Järvinen, H., Koranyi, L., Bourey, R., Lindström, J., Nikula-Ijäs, P., & Groop, L.C. (1994). Effect of insulin on GLUT-4 mRNA and protein concentrations in skeletal muscle of patients with NIDDM and their first-degree relatives. *Diabetologia, 37*(4), 401-407.

Schrauwen-Hinderling, V.B., Kooi, M.E., Hesselink, M.K.C., Jeneson, J.A.L., Backes, W. H., van Echteld, C.J.A., & Schrauwen, P. (2007). Impaired in vivo mitochondrial function but similar intramyocellular lipid content in patients with type 2 diabetes mellitus and BMI-matched control subjects. *Diabetologia, 50*(1), 113-120.

Shepherd, P.R., & Kahn, B.B. (1999). Glucose transporters and insulin action: Implications for insulin resistance and diabetes mellitus. *New England Journal of Medicine, 341*(4), 248-257.

Talanian, J.L., Holloway, G.P., Snook, L.A., Heigenhauser, G.J., Bonen, A., & Spriet, L.L. (2010). Exercise training increases sarcolemmal and mitochondrial fatty acid transport proteins in human skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism, 299*(2), E180-E188.

Thomas, N. & Martin, J.C. (2009) Single leg cycling: An evaluation of pedal power. *Medicine and Science in Sports and Exercise, 41*(Suppl 1), 54–55.

Zanuso, S., Jimenez, A., Pugliese, G., Corigliano, G., & Balducci, S. (2010). Exercise for the management of type 2 diabetes: A review of the evidence. *Acta diabetologica, 47*(1), 15-22.

Zierath, J.R., Krook, A., & Wallberg-Henriksson, H. (2000). Insulin action and insulin resistance in human skeletal muscle. *Diabetologia, 43*(7), 821-835.